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Substrate-induced volatile organic compound emissions from compost-amended soils

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Abstract The agronomic effects of composts, mineral fertiliser and combinations thereof on chemical, biological and physiological soil properties have been studied in an 18-year field experiment. The present study aimed at tracing treatment effects by evaluating the volatile organic compound (VOC) emission of the differently treated soils: non-amended control, nitrogen fertilisation and composts (produced from organic waste and sewage sludge, respectively) in combination with nitrogen fertiliser. Microbial community structure was determined by denaturing gradient gel electrophoresis (DGGE). Aerobic and anaerobic soil VOC emission was determined after glucose amendment using proton transfer reaction–mass spectrometry (PTR-MS). After inducing VOC production by substrate (glucose) addition and at the same time reducing oxygen availability to impair degradation of the produced VOCs, we were able to differentiate among the treatments. Organic waste compost did not alter the VOC emissions compared to the untreated control, whilst sewage sludge composts and mineral fertilisation showed distinct effects. This differentiation was supported by DGGE analysis of fungal 18S rDNA fragments and confirms earlier findings on bacterial communities. Three major conclusions can be drawn: (1) VOC patterns are able to discriminate among soil treatments. (2) Sewage sludge compost and mineral fertilisation have not only the strongest impact on microbial community

composition but also on VOC emission patterns, but specific tracer VOCs could not be identified. (3) Future efforts should aim at a PTR-MS-linked identification of the detected masses.

Keywords VOC · PTR-MS · Compost · Soil microbial community structure

Introduction

Permanent agricultural use of soil decreases nutrient and soil organic matter contents (Chan et al. 2002). To maintain plant nutrient contents, in most cases, mineral fertilisers are used, whilst the role of soil organic matter as an essential determinant of soil fertility and stability is often underestimated (Ros et al. 2006a). To build up soil organic matter in arable soils—a process much slower than its degradation—modern farming approaches include a sustainable management of soils to maintain the long-term productivity. Amongst the options is to reduce the use of mineral fertilisers and replace them by organic fertilisers and/or composts, often produced from agricultural or domestic residues (Garcia et al. 1994; Pascual et al. 1997). In particular, soils with a low soil organic matter (SOM) content (e.g. below 2%) benefit from organic matter input (Lal 2005; Roldan et al. 2005; Ros et al. 2006a). Besides this, conversion of organic residues such as sewage sludge, green cut, organic wastes or animal manure into useable composts reduces the amount of waste deposited into landfill sites, thereby improving the cycling of matter. Besides these known benefits, soil compost and fertiliser amendments affect the soil microbial community composition and activity in different ways, as has been shown by Innerebner et al. (2006) and Ros et al. (2006a,

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b). Microbiological tools range from conventional cultivation approaches to molecular techniques, each of them with its own advantages, biases and limitations. Identification and characterisation of the microbial community from differently treated arable soils can help to detect sources for human and plant pathogens and is thus needed for the regulation of compost application.

Microorganisms are known to produce a great variety of different compounds. Many of those are volatile, which is making them easily accessible for detection prior to any visible signs of microbial growth. The detection of volatile organic compounds (VOCs) by various approaches has long been practised (e.g. Brown 1922 cited in Linton and Wright 1993), but recently, new detection methods have increased the potential of such studies. In addition to molecular tools targeting microbial community structure, VOCs may help to detect the presence of certain microorganisms as microbial VOCs emerge from various habitats like soil (Schade and Custer 2004; Asensio et al. 2007a), litter (Leff and Fierer 2008), food (Börjesson et al. 1990, 1992; Kersh et al. 1998; Schnürer et al. 1999; Gao and Martin 2002; Mayr et al. 2003), composts (Wheatley et al. 1997; Fischer et al. 1999; Smet et al. 1999), buildings (Wilkins et al. 2000; Fischer and Dott 2003; Matysik et al. 2008), plant surfaces (Tirranen and Gitelson 2006), domestic biowaste (Mayrhofer et al. 2006), arctic ice (Dickschat et al. 2005a), seawater (Dickschat et al. 2005b) and air conditioning systems of cars (Rose et al. 2000). Volatile organic compounds is a broad term defining a large group of compounds, including metabolites produced by all life forms. It has been suggested by several authors that some microorganisms may produce specific VOCs, making it possible to determine their identity by detecting certain VOCs (Isidorov and Jdanova 2002; Smolander et al. 2006; Bunge et al. 2008).

Proton transfer reaction mass spectrometry (PTR-MS) allows the online measurement of VOCs down to the parts per trillion by volume (pptv) level (Hansel et al. 1995, 1998; Lindinger et al. 1998). The main advantage of this method is the soft ionising reaction (a proton transfer from H_3O^+ directly to the compounds) which uses low reaction energy and therefore reduces the number of gas molecules that get fragmented. The abundance of any trace gases measured has to be determined indirectly via the co-measurement of standards (trace gases of known amount) as the PTR-MS detects any trace gas as m/z (mass-to-charge ratio) via an electron multiplier. Also, the identification of any compound is restricted to the analysis of the mass (most often assumed to be $(m/z - 1)$) and the mass spectra (if available) of any compound. Another advantage of the PTR-MS method is the ability to measure online, allowing a more detailed view on the temporal dynamics of microbial VOC production (Bunge et al. 2008).

The aims of this study were to evaluate if the different amendments (mineral N fertiliser, municipal organic waste compost and sewage sludge compost) could be distinguished by VOC emission patterns as well as elucidating types and amounts of VOCs produced by the soils. Besides the fact that PTR-MS has been used for the determination of soil-derived VOCs only by a few scientists so far (Ezra et al. 2004; Schade and Custer 2004; Asensio et al. 2007a, b), another innovative concept of this study was the use of anaerobic and substrate-induced approaches for VOC measurements. As suggested by several authors (Larsen and Frisvad 1995a, b; Fischer et al. 1999; Fiedler et al. 2001), VOCs could be used to identify certain fungi back to species level. Therefore, our second objective was to link specific VOCs to changes in the composition of fungal communities determined by denaturing gradient gel electrophoresis (DGGE).

Materials and methods

Soil samples were taken at the research site “Ritzlhof” near Linz, Upper Austria. The site is part of a crop rotation fertilisation experiment (grain maize, summer wheat and winter barley) that was started in 1991 by the Austrian Agency for Health and Food Safety. The soil is a loamy silt with a pH of 6.8 (± 0.4), an organic matter content of 1.9% (Ros et al. 2006a; Innerebner et al. 2006) and a water holding capacity of 0.5 g H_2O per gram dry soil. Soil characteristics and heavy metal contents are described in Ros et al. (2006a). Compost amendment takes place once a year in spring or autumn, followed by tilling (20 to 40 cm depth) shortly before crop planting. Each fertilisation treatment was applied on four randomly distributed replicate plots (6×5 m each). For this investigation, the following treatments were selected: (1) unfertilized control (control), (2) mineral fertiliser (ammonium nitrate, NH_4NO_3 , applied at 80 kg N per hectare; 80N), (3) combined amendment of mineral fertiliser (80 kg N per hectare) plus one of two different composts corresponding to a total of 175 kg N per hectare: urban organic waste compost (OWC+80N) and sewage sludge compost (SSC+80N).

Soil samples were collected in October 2008 prior to seeding. To obtain representative samples, five randomly taken soil cores (1- to 15-cm depth) per plot were pooled to give one sample. The samples were stored at 4°C overnight. Small aliquots of samples for DNA analysis were kept frozen at -20°C.

The samples for VOC analysis were sieved (grid size 2 mm), divided into portions of 100 g (equal to ~80 g dry weight) and further stored at 4°C. Prior to infrared gas analysis (IRGA) or PTR-MS measurement, the samples

were equilibrated to room temperature 2 h before the measurement started.

Water holding capacity (WHC), dry weight and water content were estimated gravimetrically. For estimating the soil moisture at 100% WHC, glass tubes were filled with soil, saturated with distilled water and allowed to drain for 2 h on silica sand (Leff and Fierer 2008).

DNA extraction was performed using the PowerSoil DNA Isolation Kit (MoBio Laboratories Inc., USA) according to the manufacturer's protocol, with three alterations: (1) Instead of using the FastPrep instrument for high-speed shaking, the tubes were shaken horizontally on a MM2000 shaker (Retsch, Haan, Germany) for 15 min at 60 rpm. (2) After addition of solution C5 (step 16), the tube was centrifuged for 1 min instead of 30 s. (3) Instead of eluting the DNA bound to the membrane with 100 µl solution C6 (step 20), 50 µl of 60°C warm DNase-free water was used twice and collected in two different Eppendorf tubes. DNA yield and quality were assessed by electrophoresis on a 1.0% (w/v) agarose gel followed by DNA concentration measurements using PicoGreen dsDNA quantitation reagent (Invitrogen, USA).

Extracted DNA was amplified in a PCR thermocycler (FlexCyclerTM, Analytik Jena AG, Germany). Each PCR mixture had a reaction volume of 25 µl containing 1.25 U BioThermTM DNA Polymerase (genXpress, Austria), 200 µM dNTP mix, 200 nM of each primer, 20 µg BSA (301 nM), 40 µg DMSO, 1 mM MgCl₂, 1× PCR buffer (genXpress) and 1–10 ng of template. For amplification of the fungal 18S rDNA, the primers FR1-GC (CCC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GCC GAI CCA TTC AAT CGG TAI T) and FF390 (CGA TAA CGA ACG AGA CCT; Vainio and Hantula 2000) were used. The PCR programme was conducted with an initial denaturation step at 95°C for 8 min, followed by 35 thermal cycles of 30 s at 94°C and 45 s at 48°C, and 3 min at 72°C and a final extension step at 72°C for 10 min were chosen. For amplification of the fungal rDNA internal spacer (ITS) region DNA fragments, a semi-nested approach as described by Bastias et al. (2006) was used. PCR was conducted using a FlexCyclerTM PCR thermocycler (Analytik Jena AG), and products were checked by electrophoresis in 1.5% (w/v) agarose gels following ethidium bromide staining (10 mg ml⁻¹), as well as quantified using PicoGreen dsDNA quantitation reagent (Invitrogen).

DNA fragments were subjected to DGGE (Muyzer et al. 1993). The acryl amide (7%) gel with a 30–60% denaturing gradient (100% denaturant is 7 M urea plus 40% formamide in 1× TAE) was run for 16 h at 60°C and 100 V on an IngenyPhorU2 system (Ingeny International BV, the Netherlands).

Gels were stained with silver nitrate (Sanguinetti et al. 1994) using an automated gel stainer (Hoefer, Amersham

Pharmacia Biotech, USA). The stained gels were photographed, dried and scanned.

Images of the gels were analysed by the software package GelComparII, version 4.0 (Applied Maths, Ghent, Belgium). The bands were designated manually to be able to exclude artefacts deriving from gel staining or imaging. Prior to cluster analysis, the band matching task was deployed to align bands from different lanes to one certain position. Band matching was performed using an optimisation value of 1% and a position tolerance of 1%. Similarity calculation was based on the Dice coefficient (Legendre and Legendre 1998) and resulted in a distance matrix, visualised in dendrograms calculated using the Ward algorithm (Legendre and Legendre 1998).

Basal respiration was determined by CO₂ evolution from sieved (Ø 2 mm), moist soil samples (50% of maximal WHC) at 22°C after a 6-h pre-incubation period with a continuous flow IRGA system (Heinemeyer et al. 1989). Plastic tubes were filled with 100 g moist samples, and CO₂ production was measured hourly for 15 h under permanent humid air flow. All data are given on a soil dry mass basis.

Microbial biomass carbon (C_{mic}) was determined by substrate-induced respiration. Forty grams (DM) of moist soil was mixed with 1% (w/w) glucose (Anderson and Domsch 1978), and the substrate-induced CO₂ production rate was determined 6 h after glucose amendment (Heinemeyer et al. 1989).

To test if the different soil treatments had an impact on C_{mic} or basal respiration rates, analysis of variance (ANOVA, $p < 0.05$) was performed with SPSS version 15.0 (SPSS Inc., USA). Homogeneity of variances was tested with Levene test, and post hoc analysis was performed with Tukey HSD test.

For the PTR-MS and PTR-time-of-flight (TOF)-MS measurements (Lindinger et al. 1998), approximately 100 g of samples was filled in Teflon cylinders. Preliminary tests showed that only after addition of could glucose measurable VOC concentrations be obtained. In addition, the number of detected VOCs was considerably higher for anaerobic (using N₂ for purging) than aerobic conditions. Thus, this setup was chosen for the present study. The cylinders were kept in the dark and in a water bath to maintain a constant temperature of 25°C. Four samples were analysed per batch. All cylinders were purged with 70 ml min⁻¹ N₂ for anaerobic measurements. During the measurement, the cylinder under investigation was connected to the PTR-MS for approximately 10 min. The influx gas was humidified using a water-filled bottle (bubbler) before passing through a charcoal filter and a platinum catalyst (400°C) to remove all VOCs from the carrier gas. Each sample was screened hourly for several

masses between 18 μ and 200 μ for a period of at least 40 h after glucose amendment.

The PTR-MS drift tube was operated at 2.2 mbar and at 60°C. The drift voltage was 600 V giving an E/N (electric field strength across the reaction chamber to buffer gas number density within the chamber) of approximately 120 Td (1 Td = 10×10^{-17} V cm²).

Volatile organic compound identification was performed using the molecular weight data from PTR-MS and PTR-TOF-MS measurements. Possible sum formulas were calculated using Molecular Fragment Calculator 1.0 (© James E. Deline, Windows Version by Dwight L. Smith). Molecular conformations were aligned using NIST Standard Reference Database Number 69.

The PTR-MS-derived datasets were normalised so the unit of abundance was normalised counts per second (ncps). All masses deriving from water clusters (19, 21, 37, 39 and 55) were eliminated. ANOVA and post hoc tests were performed using SPSS version 15.0 (SPSS Inc.). Calculations were performed using the time chart dataset for all detected masses as well as curve parameters such as the absolute peak height (data not shown). To summarise differences in VOC emissions between the different treatments, principal component analysis (PCA) was calculated from total VOC abundance data of all relevant masses (except water clusters). The PCA data were visualised using a multidimensional scaling approach (Fig. 6) where the distances between the objects (replicates of the different treatments) were calculated as Euclidean distances.

Results

Analysis of microbial community structure by PCR-DGGE

The DGGE fingerprints of the ITS region from the different samples and replicates appeared not to be very different (Fig. 1). All samples contained a high number of bands in the upper and middle sections of the gel and a smaller number of bands in the high-GC section of the gel. Nevertheless, analysis of banding patterns resulted in the formation of three main clusters: 80N and SSC+80N replicates grouped separately from the cluster formed by OWC+80N and control samples that formed two subclusters with a similarity of 76%. The diversity between replicates of any treatment was relatively high, ranging from 78% to 95%.

The DGGE fingerprints of the 18S rDNA (data not shown) showed similar groupings. However, differences within the groups were higher and showed similarities from 83% to 97%. Most bands occurred in different intensities, but were present in almost every lane. Control and OWC+80N samples showed a similarity of

65%, whilst the SSC+80N and 80N samples grouped more distantly.

Basal respiration rates and microbial biomass

Sewage sludge compost-amended samples showed the highest basal respiration and microbial biomass, 0.7 $\mu\text{g CO}_2$ per gram soil per hour and 75 $\mu\text{g C}_{\text{mic}}$ per gram soil, respectively. The samples collected in October 2008 showed significant differences between control and OWC+80N, control and SSC+80N, 80N and OWC+80N as well as 80N and SSC+80N. In other words, the compost-amended soils differed significantly from the control and the mineral fertilised soil as they had higher microbial biomass contents and basal respiration (Fig. 2).

For all treatments, microbial biomass ($r=0.60$, $p<0.05$) and basal respiration ($r=0.61$, $p<0.05$) were positively correlated with total VOC production.

VOCs detected and compound identification

When samples were not amended with glucose, the VOC emissions of the soil samples were below the detection limit of the PTR-MS. The results discussed below thus refer only to measurements after glucose amendment. Under anaerobic conditions, a considerable number of masses (39) showed high abundance (>10 ncps; Fig. 3). The masses with the highest abundance ($>10^3$ ncps) under anaerobic conditions were m/z 47, 59, 57, 45, 43 and 41. In contrast, under aerobic conditions, only four masses (m/z 59, 45, 33 and 47) exceeded 10 ncps.

All masses detected with PTR-MS under anaerobic conditions range from m/z 30 to m/z 120. The six masses exceeding 10^3 ncps were chemically identified or at least limited to certain sum formulas. For mass 57, four different compounds matched the identified sum formula: propenal or methylketene ($\text{C}_3\text{H}_4\text{O}$) and butene or propene (C_4H_8). Both masses 43 and 41 did not show any feasible match, but molecules of higher weight tend to break up in fragments of this size, e.g. acetic acid, propanol or propandiol. Mass 47 would be that of ethanol and formic acid, being both end products of the anaerobic energy chain. Mass 59 would be due to acetone, whereas mass 45 would be that of three potential compounds: acetaldehyde, propane and vinyl alcohol; however, acetaldehyde seems to be the most logic candidate as it is formed as intermediate product of ethanol degradation.

Volatile organic compound emission patterns and dynamics

Due to its limited compound identification capabilities, specific masses cannot be unambiguously assigned to a certain substance. However, some masses could at least be

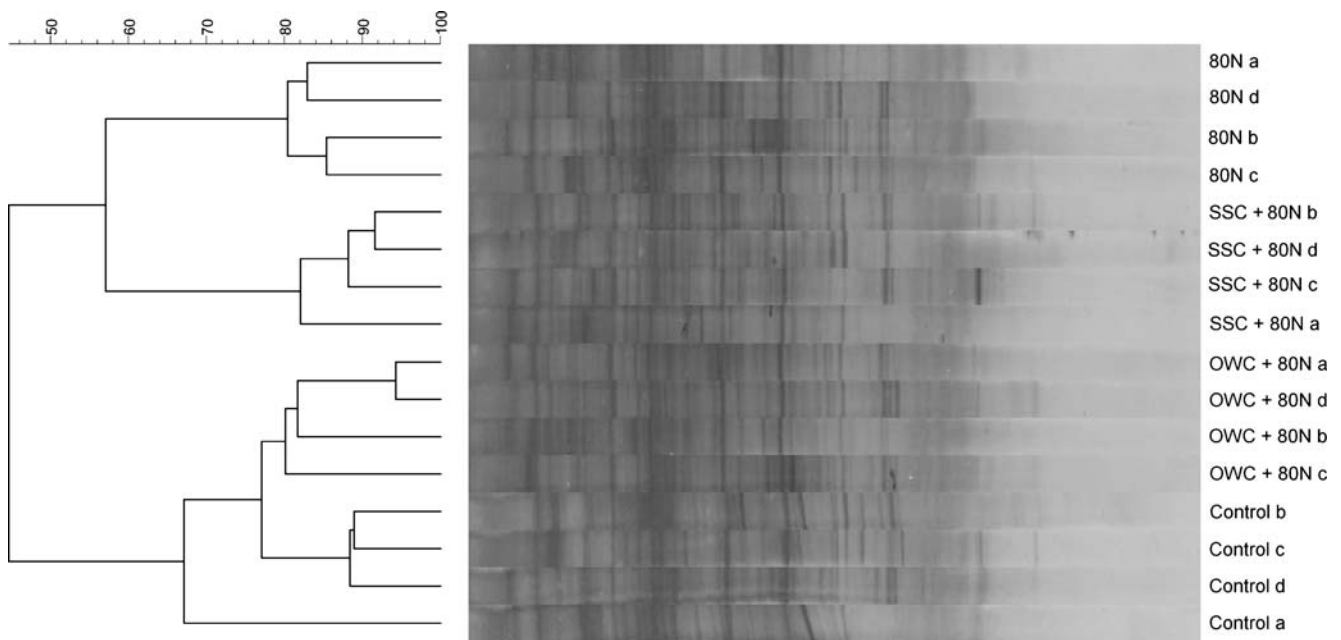


Fig. 1 Cluster analysis of DGGE fingerprints based on fungal ITS DNA fragments from four replicates ($n=4$) and bars of four differently treated soils (non-amended control: control; mineral

nitrogen-amended: 80N; urban organic waste compost plus mineral fertiliser: OWC+80N and sewage sludge compost plus mineral fertiliser: SSC+80N)

tentatively identified. Volatile organic compound emission patterns (maximum peak height of all masses found) varied among the different treatments (Fig. 4). The patterns of the control and OWC+80N samples were quite similar, whilst the 80N and the SSC+80N samples deviated considerably. The SSC+80N samples showed the highest number of different masses. Compared to the SSC+80N samples, masses 72, 77, 85 and 88 were absent in all control replicates, and mass 72 and 85 were missing in all OWC+80N replicates. But there were also masses missing in, one, two or three replicates of a treatment only, like masses 51, 66, 93, 101 and 103, which were missing in some of the

control replicates (Fig. 4). Similarly, masses 62, 77, 101 and 103 were missing in some OWC+80N replicates, and masses 72, 77, 85 and 88 were below the limit of detection in some 80N replicates.

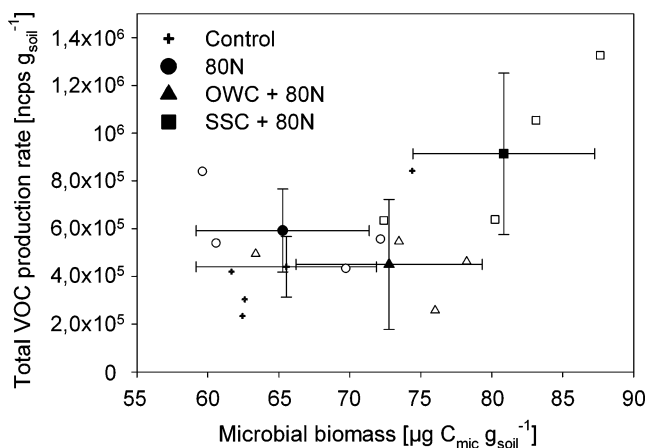


Fig. 2 Comparison of the total VOC production rate and the microbial biomass (C_{mic}) of four differently treated soils. The values are means over all replicates ($n=4$). Bars are standard deviations

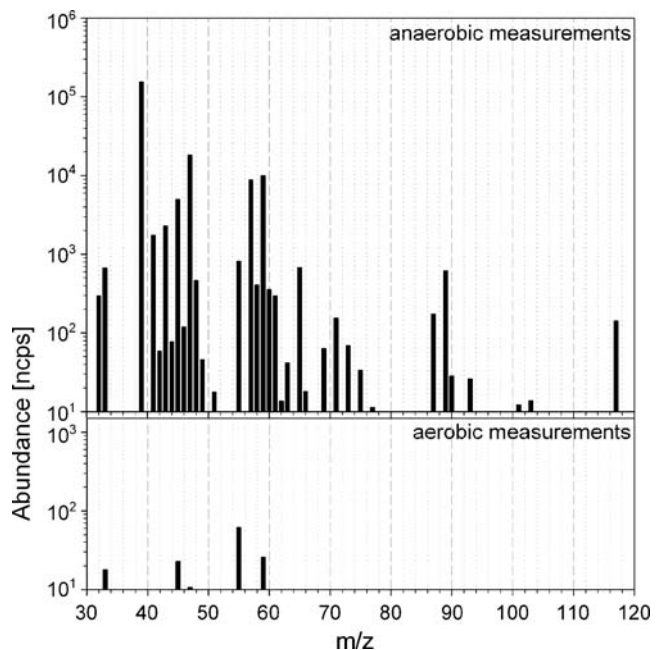


Fig. 3 Comparison of measurements under anaerobic and aerobic conditions. The abundance (ncps normalised counts per second) of different masses (m/z mass-to-charge ratio) are shown here. Measurements took place under anaerobic and aerobic conditions. The values shown here are the average of peak maximums of all four treatments and its parallels. All ncps values correspond to 100 g soil

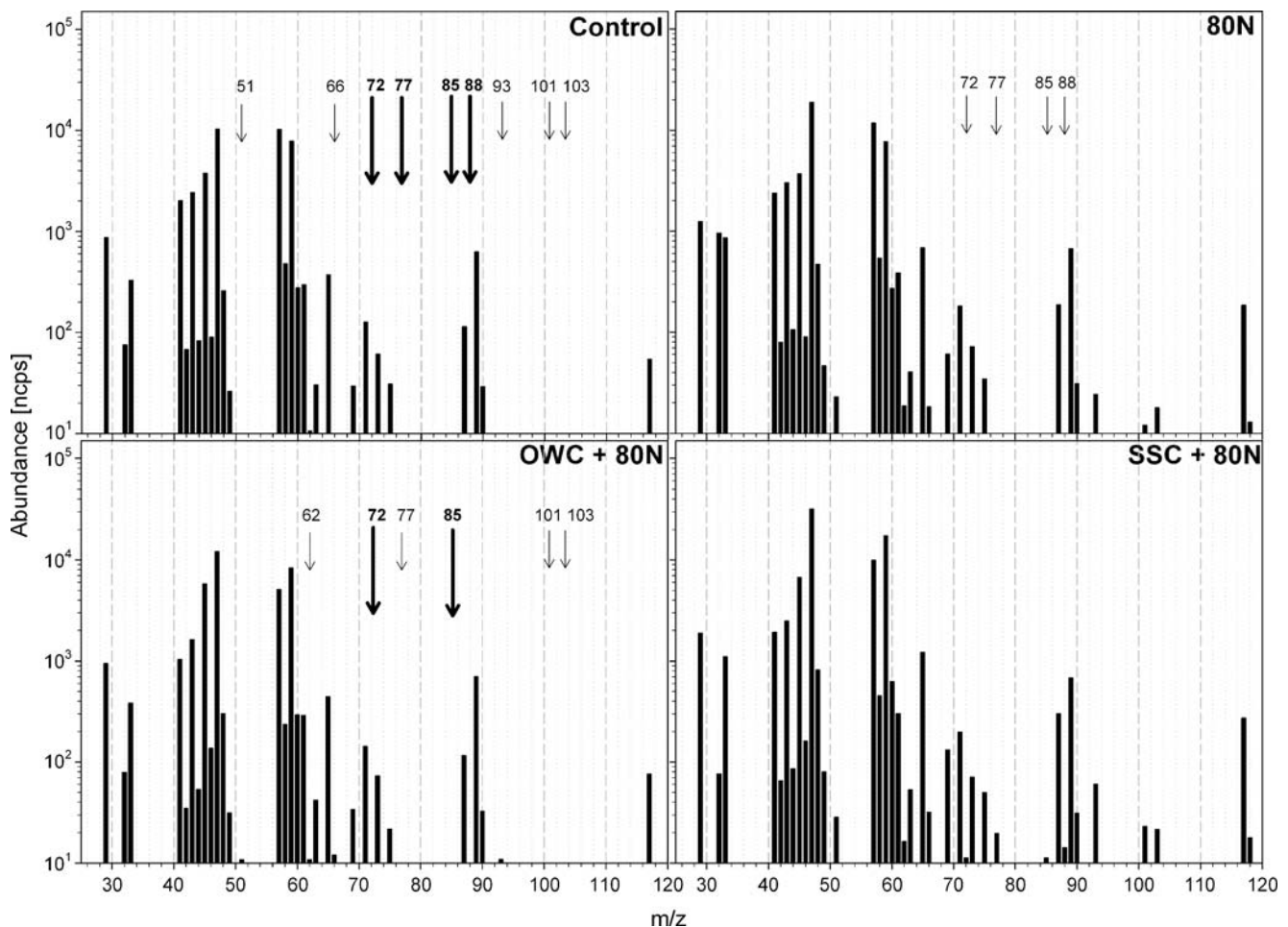


Fig. 4 Mass spectra of four different treatments control, (80N) mineral N fertiliser, (OWC+80N) mineral N plus urban organic waste compost and (SSC+80N) mineral N plus sewage sludge compost-amended samples, determined under anaerobic measurement conditions. The abundances are given as maximum ncps of averaged

parallels for each mass (m/z) that occurred with more than 10 ncps. All ncps values are corresponding to 100 g soil. Missing masses are indicated with *bold arrows*. Masses below the detection limit in some replicates are labelled with *arrows*

Temporal VOC charts revealed differences between the different treatments as well as between different masses (Fig. 5). The abundance of certain masses varied strongly within the replicates of one treatment, but the shapes of the curves were similar. For example, the SSC+80N replicates of m45 show very similar time progression with a VOC production starting at 15 h, a maximum at 26 h and a final decrease until 40 h after glucose amendment (Fig. 5). The time chart for m47 looks different as the production starts at 14 h after glucose amendment, thus not even reaching a maximum within 40 h. The treatments differed markedly in VOC abundance and in the shapes of the curves.

ANOVA over all time points, treatments and masses did not show any significant differences at all. However, ANOVA using maximal abundances of all masses showed significant differences among the treatments. Principal component analysis over all masses that showed significant differences between treatments resulted in the formation of

three groups (Fig. 6); SSC+80N and 80N samples were clearly separated from the control and the OWC treatments. The placement of SSC+80N samples towards the positive end of the plot along dimension 1 indicates that these samples produced the masses contributing to dimension 1 in highest amounts. The placement of the other samples in the biplot allows similar interpretations concerning the production of VOCs for all samples.

Discussion

Analysis of microbial community structure by DGGE

DGGE fingerprints from ITS and 18SSU rDNA fragments appeared to be complex, reflecting the high diversity of soil fungi. Most bands occurred in all lanes, differing slightly in their intensity, indicating the soil fungal community to be

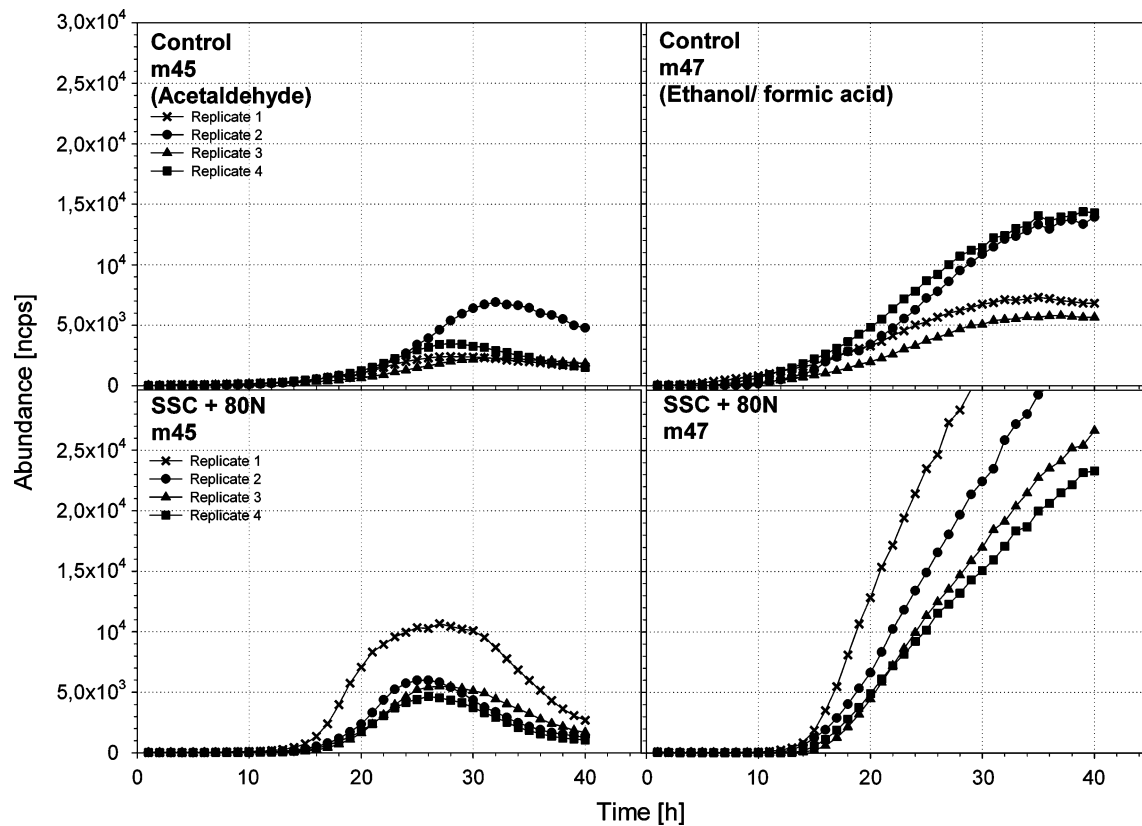


Fig. 5 Exemplary temporal charts of m45 (acetaldehyde) and m47 (ethanol and formic acid) of control and SSC+80N replicates. The net VOC production rates of the replicates are varying considerably, but the curve shapes are similar within the treatment (control or SSC+

80N). The curve shapes of m45 and m47 are different; SSC+80N samples were found to produce more VOC, and in case of m45, the maximum was reached faster

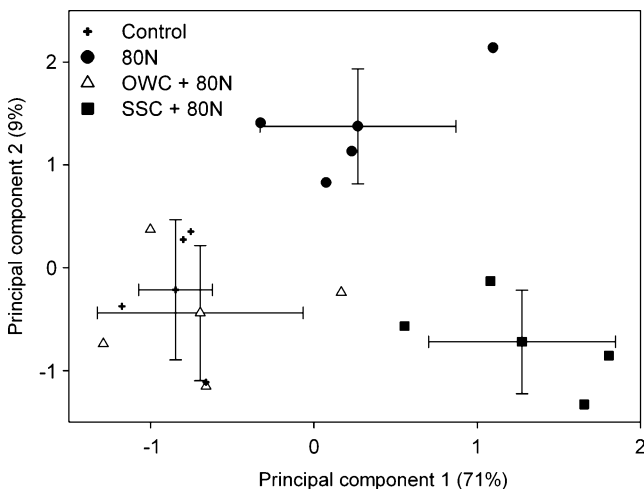


Fig. 6 Volatile organic compound emission patterns of differently treated soils visualised by a multidimensional scaling (MDS) diagram. Principal components of all significant masses are shown as dimensions 1 and 2 (cumulatively, explaining 80% of the variance). Group means and standard deviations are given

relatively stable against treatment-driven influences. Differences in DGGE fingerprints were due to the occurrence of a few faint bands which were missing in other lanes, again denoting the weak influence of the different treatments. These findings confirm the results of Ros et al. (2006b) on the bacterial community structure of the same soil and treatments using 16SSU rDNA DGGE fingerprint analysis and of Innerebner et al. (2006) who found differences in ammonia-oxidising bacteria community structure.

Cluster analysis of both ITS and 18SSU fingerprints revealed highest similarities between the community structure of control and OWC+80N samples. 80N samples and SSC+80N samples clustered separately, indicating a stronger influence of these treatments on fungal community. An explanation may be that OWC+80N is (despite the nitrogen fertiliser content) a substrate similar to natural organic carbon sources (Crecchio et al. 2001; Pérez-Piqueres et al. 2005), whilst mineral fertiliser alone or SSC+80N are different from common organic matter sources and thus to a larger degree affect the microbial community structure (Tilman 1998; Pascual et al. 1997; Six et al. 1999; Kandeler et al. 2000). This may be linked to a slower nutrient release compared to mineral fertiliser and sewage sludge compost

or to the lower heavy metal load of organic waste compared to sewage sludge compost. Mature organic waste compost contains predominantly organically bound nitrogen and stable organic matter, most of it only poorly accessible to microorganisms, somehow presenting a kind of natural amendment. It has been reported earlier that some soil treatments (e.g. municipal organic waste compost) take weaker influence on the composition of soil microbial community structure than edaphic conditions, temperature or soil depth (Crecchio et al. 2001; Pérez-Piqueres et al. 2005; Innerebner et al. 2006; Ros et al. 2006a, b). In contrast, the application of sewage sludge compost, consisting of faeces and wastewater-derived substances is an artificial intrusion in the soil ecosystem, supplying soil with unfamiliar compounds and nutrients. Similarly, the application of mineral fertilisers may change the substrates nutrient availability and as a consequence also microbial community structure of soils. Additionally, mineral fertilisers and sewage sludge composts are known to release nitrogen very fast (Insam and Merschak 1997; Tilman 1998; Pascual et al. 1997; Six et al. 1999), resulting in changes of microbial community structure.

Basal respiration and microbial biomass

Microbial biomass and basal respiration rates differed significantly between the control and SSC+80N treatments (Fig. 2). Compost-amended samples differed from non-compost-amended samples, showing elevated soil microbial activity and C_{mic} . Compost amendments contribute to the buildup of SOM (Lal 2005; Roldan et al. 2005; Ros et al. 2006a) and change the microbial degradability and the diversity of organic substrates, resulting in an increased microbial biomass and diversity (Insam 1990; Carter 1996). In contrast, mineral fertiliser amendment can deplete soil organic matter (Six et al. 1999; Ros et al. 2006a, b).

Our C_{mic} and basal respiration rates were rather low in comparison with those of previous studies (Ros et al. 2006a, b), probably because our samples were taken in autumn when soil microbial activity usually declines (Bardgett et al. 1999), whereas Ros et al. (2006b) sampled their soils in spring. Our results confirm those by Ros et al. (2006b) who were able to show differences in C_{mic} and basal respiration among the different treatments and a general decrease of these properties with soil depth. Another explanation for the lower C_{mic} may be soil management effects since our samples were taken 7 days after ploughing, which changes the accessibility of organic matter and oxygen to the microbes (Stockfisch et al. 1999; Balesdent et al. 2000). Ploughing also mixes the lower soil layers low in microbial biomass with the upper soil layers. Despite OWC+80N and control samples differing in microbial biomass values, their VOC production rates were

similar (Fig. 2); it may be concluded that an increased VOC production in the OWC+80N samples may have been accompanied by an increased consumption of VOCs. DGGE fingerprint analysis revealed high similarity between the control and OWC+80N samples. In contrast, control and 80N samples showed similar C_{mic} but differed in VOC production rates. An explanation may be the induction of a priming effect that liberates carbon compounds from the indigenous soil pool (Blagodatskaya and Kuzyakov 2008).

Microbial growth and VOC production

Along with plant roots, microorganisms have been reported to be a major source of VOCs produced in soils (Stotzky and Schenk 1976; Stahl and Parkin 1996; Mackie and Wheatley 1999; Isidorov and Jdanova 2002; Leff and Fierer 2008; Kai et al. 2009; Insam and Seewald 2010). In contrast to this and despite microbial VOC production, plain soil (without plant roots) is reported to be a VOC sink, strongly dependent on soil temperature and moisture (Asensio et al. 2007a). Volatile organic compound sorption to clay minerals (Petersen et al. 1995; Chen and Wu 1998; Ruiz et al. 1998; Poulsen et al. 1998; Serrano and Gallego 2006), solubilisation in the soil aqueous phase (Asensio et al. 2007a) or degradation by other microorganisms (Cleveland and Yavitt 1998; Smolander et al. 2006; Owen et al. 2007; Insam and Seewald 2010) may explain any soil net VOC uptake or variations between replicates (Leff and Fierer 2008). Microbial VOCs may derive from many different metabolic sources, such as primary metabolism like end products of fermentative pathways such as ethanol (ethanol fermentation), butyric acid and acetone (butyric acid fermentation) and as volatile intermediates of aerobic or anaerobic detritus decomposition (Castaldelli et al. 2003; Karl et al. 2003). More complex VOCs, with a higher molecular weight, may derive from secondary metabolism pathways, such as production of signalling compounds or antibiotics (Mathivanan et al. 2008; Splivallo 2008). The low VOC emissions from the OWC+80N samples (which were comparable to the VOC emissions of the control) may be due to a fast degradation of VOCs.

Detection and identification of VOC

Under anaerobic conditions, a much higher number and abundance of masses could be observed after glucose addition than under aerobic measurements or non-glucose-amended tests. This may be due to the fact that glucose addition-induced microbial activity entails fermentation of glucose and SOM. The end products of anaerobic fermentation (such as ethanol, methanol, butyric acid, acetone and formic acid) are highly volatile compounds produced rapidly in high amounts. In contrast, under aerobic

conditions, the most efficient way of producing energy from glucose and soil substrates is aerobic respiration resulting in the release of carbon dioxide and water. The production of many other VOCs would start with the secondary metabolites. In comparison to the end products of the energy chain, secondary metabolites are produced in much lower amounts, thus being diluted in the steady flow of the PTR-MS carrier gas used in this investigation. Furthermore, VOCs produced under aerobic conditions may at the same time serve as energy source for VOC-degrading microorganisms (soil microbial food web), resulting in VOC mineralisation (Cleveland and Yavitt 1998; Owen et al. 2007).

Four masses were identified: m33 as methanol, m45 as acetaldehyde, m47 as formic acid and ethanol and m59 as acetone. For all other masses, literature information considered and additional PTR-TOF-MS measurements carried out suggest a tentative mass classification. As isotopes, multiple ionisation, molecule fragmentation and water or ion clustering lead to mass overlap, identification of masses from complex systems as soils remains a sophisticated task. The combined application of PTR-MS with TOF-MS helps to determine the molecular mass of the respective VOC to the first two decimal places. Therefore, it is possible to limit the chemical compound to its mass formula. Another option is to make use of the isotopic pattern of elements. Then, mass 48 appeared proportional to mass 47 ($2.3 \pm 0.38\%$), probably due to the presence of the carbon isotope ^{13}C (with a natural abundance of 1.1%). For example, one molecule of ethanol contains two C atoms, leaving two possible positions for the integration of a ^{13}C isotope, giving a total chance of 2.2% for an incorporation of ^{13}C . The remaining 0.1% may result from the incorporation of ^2H ($6 \times 0.0115\%$) and ^{17}O ($1 \times 0.038\%$). Previous investigations showed that compounds derived from molecular fragmentation contributed to masses 41, 43 and 69 in high concentrations (Mayr et al. 2003; Yeretizian et al. 2003; Ezra et al. 2004; Steeghs et al. 2004; Mayrhofer et al. 2006; Araghipour et al. 2008). In this investigation, these masses were also found in considerable amounts.

To date, only a few studies concerning VOC production from soil microorganisms have been conducted. Many of these studies applied GC-MS approaches with headspace accumulation techniques, thus mainly investigating VOCs with molecular weights $>100 \mu$, like limonene or pinene (Isidorov and Jdanova 2002; Asensio et al. 2007a; Leff and Fierer 2008; Insam and Seewald 2010). Many of these “heavy” VOCs are known to be intermediate products of plant residue degradation or secondary metabolites of microorganisms. In this investigation, with the aid of PTR-MS, masses below 100μ were detected in highest concentrations, some of the VOCs identified as end products of glucose fermentations (like ethanol, acetaldehyde or

acetone). Therefore, we suppose that the VOCs measured mainly derived from glucose fermentation pathways, induced by glucose amendments. Thus, the applied measurement method reflects the microbial metabolism during anaerobic glucose fermentation.

Emission dynamics and comparison of VOC emission pattern

The absence of certain masses in the VOC emission pattern of the different treatments could be due to production rates yielding concentrations lower than the detection limit of PTR-MS (5 pptv). Mass spectra of SSC+80N samples showed the highest number of masses (39, Fig. 4) and the highest VOC concentrations. The VOC emission patterns of the treatments OWC+80N and control were the most similar. The differences in the dynamics of VOC production among samples may be attributed to several reasons. One possible explanation for the more rapid increase in the SSC+80N samples compared to the control samples (Fig. 5) may be a shift of the microbial community from r- to K-strategists. On the other hand, it may also indicate a delayed response of VOC degraders in the case of the SSC+80N samples. In any case, also these data support earlier findings that sewage sludge amendments have a strong impact on biological and biochemical processes in soil (e.g. Ros et al. 2006a).

Significant differences in peak maxima of some VOCs occur between treatments. Principal component analysis of these masses visualised with multidimensional scaling resulted in a Euclidean distance matrix which showed the separation of the treatments into three clusters (Fig. 6). Here, 80N and SSC+80N samples clearly separated from each other and from OWC+80N and control samples. Interestingly, it was not possible to distinguish between OWC+80N and control samples using PTR-MS measurements. This may be due to the high similarity in the fungal community structure of these two treatments as denoted by DGGE fingerprint analysis where control and OWC+80N samples clustered (Fig. 1).

Conclusion and outlook

Differences in overall soil VOC emission patterns could be detected by PTR-MS measurement, showing the power of PTR-MS of being a valuable tool for the online monitoring of certain metabolic activities of soil microbial communities. However, the online measurement requires the steady flow of carrier gas, and this can lead to the dilution and enhanced decomposition of the VOC trace gases. In this investigation, we tried to overcome this problem by enhancing VOC production through glucose amendment and anaerobic measurements. Greater sample volume (here

170 ml) could help to increase VOC production to measurable amounts. Direct sampling of evolved soil gas using evacuated silica-coated canes could, in future studies, help to overcome sample pretreatment and connected biases.

In our study, SSC+80N and 80N have not only the strongest impact on microbial community composition but also on VOC emission patterns. Specific tracer VOC could not be identified with the approaches used, but direct gas sampling could help to find less abundant compounds that are produced by a small group of microorganisms and under field conditions.

In this study, we were able to identify compounds occurring with masses 33 (methanol), 45 (acetaldehyde), 47 (ethanol), and 59 (acetone; confirmed by PTR-TOF-MS). Further studies should aim at identifying compounds contributing to masses like 41, 43 and 69. In this investigation, no “marker mass” could be identified for any of the four different soil treatments. Future efforts should aim at a PTR-MS-linked identification of the detected masses to be able to draw conclusions on the microorganisms and on their physiological state.

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